SPIROMICS
SubPopulations and InteRmediate Outcome Measures In COPD Study

SPUTUM PROCESSING WORKSHEET

ID NUMBER:									

FORM CODE: SPW
VERSION: 4.0 09/16/2026

Event: _____

0a) Date of Collection: ☐☐ / ☐☐ / ☐☐☐☐ 0b) Staff Code: ☐☐☐

0c) Processing Started: ☐☐:☐☐ AM_1 / PM_2

Instructions: *Complete this form while processing the sputum sample. Carefully record all data in the space provided as you move through each of the sample processing steps using MOP 5 as the guide.*

1) Weight of Entire Sample: ☐☐☐.☐☐ grams

Color and Description of Sample:

2) Salivary Contamination *(check all that apply)*:
- 2a) ☐ Minimal
- 2b) ☐ Mild
- 2c) ☐ Moderate
- 2d) ☐ Excessive

3) Consistency *(check all that apply)*:
- 3a) ☐ Watery
- 3b) ☐ Mucoid (thick, sticky, slightly frothy)
- 3c) ☐ Purulent (thick, colored, contains pus)

4) Mucus “plugs” (material that is colored and/or more opaque than clear surrounding saliva) *(check all that apply)*:
- 4a) ☐ Numerous
- 4b) ☐ Moderate number
- 4c) ☐ Sparse
- 4d) ☐ Large
- 4e) ☐ Small
- 4f) ☐ Dense/flocculent
- 4g) ☐ Diffuse opacity
- 4h) ☐ None

5) Color of plugs *(check all that apply)*:

5a) ☐ Clear
5b) ☐ White
5c) ☐ Yellow/Tan
5d) ☐ Brown
5e) ☐ Green

6) General Notes/Comments:

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6a) Sputum processing condition:

☐ Condition 1: Induced initial sample Wt. is > 1 g_1 → **Perform Steps 7-13 below**
☐ Condition 2: Induced initial sample Wt. is 0.5 g-1 g_2 → **Perform Steps 7-13 below**
☐ Condition 3: Induced initial sample Wt. is < 0.5 g_3 → **Perform Steps 7-13 below**
☐ Condition 4: Induced initial sample Wt. is < 0.5 g and a spontaneous sputum sample was acquired prior to induction$_4$ → **Perform Steps 7-8 below with induced sample; then Steps 7-13 below with spontaneous sample**
☐ Condition 5: No induced sputum sample was produced; but a spontaneous sample was acquired$_5$

6a1) Sputum processing for Condition 5:

☐ Condition A: Spontaneous sample Wt. is > 1 g_1 → **Follow Condition 1 above**
☐ Condition B: Spontaneous sample Wt. is 0.5 g-1 g_2 → **Follow Condition 2 above**
☐ Condition C: Spontaneous sample Wt. is < 0.5 g_3 → **Follow Condition 3 above**

7) Processing Whole Sample using the Mucin Method

Mucin Sample	
Weighing tray (g)	7a)
Whole sputum weight (g) ***Note: for Condition 3 and Condition 5C, use 0.100 g***	7b)
Guanidine vol. (mL)	7c)

SPIROMICS III MOP 5, Section 7.1.2. Store IMMEDIATELY in a **2-8°C refrigerator.*

8) Processing the Mucin Interactome

Osmo Sample	
Micro centrifuge tube (g)	8a)
Whole sputum weight (g) ***Note: for Condition 3 and Condition 5C, use 0.100 g***	8b)

SPIROMICS III MOP 5, Section 7.1.3. Store IMMEDIATELY in a* **-80ºC freezer.

9) Processing Total Remaining Whole Sample for Nucleotides, Cytokines, Cell Counts, Cytospins, RNA transcriptomics, and Microbiome

Weight of Centrifuge tube (g)	9a)
Weight of Sputum (g)	9b)
Volume of EDTA-DPBS added to sample (mL) ***Note: for Condition 2, Condition 3, Condition 5B, and Condition 5C skip this step***	9c)
Volume of supernatant removed (mL) (see section 10 for process) ***Note: for Condition 2, Condition 3, Condition 5B, and Condition 5C skip this step***	9d)
Volume of EDTA-DPBS added to stock sputolysin to make 0.2% DTT/Sputolysin (mL) ***Note: for Condition 2, Condition 3, Condition 5B, and Condition 5C skip this step***	9e)
Volume of 0.2% DTT/Sputolysin added to the sample (mL) ***Note: for Condition 2, Condition 3, Condition 5B, and Condition 5C skip this step***	9f)
Volume of 0.1% DTT/Sputolysin added to the sample (mL) ***Note: for Condition 1 and Condition 5A, skip this step***	9f1)
Volume of DTT containing supernatant removed (mL)	9g)
Resuspension volume of EDTA-DPBS (0.25 – 2 mL)	9h)

*******SPIROMICS III MOP 5, Section 7.1.4. Store IMMEDIATELY in a* **-80ºC freezer.**

10) EDTA-DPBS Supernatants for Nucleotides and Cytokines

Supernatants	Number of aliquots	Volume stored per aliquot (µL)
Nucleotides	10a1)	10b1)
Cytokines	10a2)	10b2)
Cytokine Zymo Research RNA/DNA	10a3)	10b3)

SPIROMICS III MOP 5, Section 7.1.4. Store IMMEDIATELY in a **-80ºC freezer.

EDTA-DTT Supernatants

Supernatants	Volume stored (mL)
Sputolysin Sup 01	10c1)
Sputolysin Sup 02	10c2)

SPIROMICS III MOP 5, Section 7.1.4. Store IMMEDIATELY in a **-80ºC freezer.

11) Cell Counts

Cell Counts:	# Dead	# Live	Total	Squamous Epithelial
Quadrant 1	11a1)	11a2)	11a3)	11a4)
Quadrant 2	11b1)	11b2)	11b3)	11b4)
Quadrant 3	11c1)	11c2)	11c3)	11c4)
Quadrant 4	11d1)	11d2)	11d3)	11d4)
Totals	11e1)	11e2)	11e3)	11e4)

SPIROMICS III MOP 5, Section 7.1.5.

Sample Concentration (cells/mL) = ([11e3 / 4] x 2 x 10^4)	11f)
Total Cell Count = (([11e3 / 4] x 2 x 10^4) x 9h)	[12f]11g)
Number of cells/weight = (11g) / weight of selected sample (9b)	[12g]11h)
Viability = (11e2 live cells / 11e3 total cells) x 100	[12h]11i)
Final Adjusted Sample Concentration (cells/mL) based on resuspension volume (range = 0.5 – 1.0 x 10^6/mL)	11j)

SPIROMICS III MOP 5, Section 7.1.5.

12) Cytospins

	# of slides stored	Amount of cell suspension used per slide (µL)
Hema 3 stained slides	1312a)	12b1) Slide 1: 12b2) Slide 2: 12b3) Slide 3: 12b4) Slide 4:

SPIROMICS III MOP 5, Section 7.1.6. DO NOT COVER SLIP THE SLIDES. Store all slides in a box at ***room temperature*.

13) RNA Transcriptomics and Microbiome samples

☐ No0

☐ Yes1

Weight of centrifuge tube (g) (from item 9a above)	13a)
Whole sputum weight (g)	13b)
Vol. of Zymo added (µL)	13c)
Vol. per aliquot (1000*13b+13c)/2 (one for RNA Trans and one for Micro)	13d)

*** SPIROMICS III MOP 5, Section 7.1.7. Store IMMEDIATELY at ***-80ºC freezer****.

1314) Comments: _____

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0e/1415) Processing Ended: ☐☐:☐☐ AM1 / PM2

END OF FORM